Effect of Methods of Platelet Resuspension on Stored Platelets

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ABSTRACT

Platelets are prepared from whole blood by differential centrifugation. Following their isolation as a platelet button, platelets are allowed to rest for a short period in the residual plasma before resuspension. In this study, the feasibility of resuspending platelets without this rest period is studied.

A total of 35 platelet concentrates (PC) were prepared from blood collected in CPDA-1 and resuspended by one of the following four methods: (1) no resting period, PC placed on a rotator immediately after preparation, (2) a 1.5 hour rest period and gentle shaking prior to rotation, (3) no rest period and immediate gentle shaking prior to rotation, and (4) a 1.5 hour resting phase and no shaking prior to rotation. Following the previous processing, all platelet concentrates were stored for 72 hours on an elliptical platelet rotator at 20 to 24°C to provide continuous agitation. A number of in vitro tests were used as indicators of platelet viability during storage. These included platelet morphology, pO2, pCO2, pH, osmotic recovery, number of platelets in the concentrate, and platelet volume distribution.

Our findings demonstrate that platelets are of similar quality after storage in all of the four groups described. Our studies suggest that the resting phase is unnecessary for platelet preparation. The elimination of the resting phase would allow platelet concentrates to be available sooner and improve cost-effectiveness of platelet preparation.

Introduction

Platelets are generally prepared from anticoagulated whole blood by differential centrifugation. The first spin produces platelet rich plasma (PRP) which is transferred to an integrally connected satellite bag. A second spin produces a unit of platelet poor plasma (PPP) and a platelet button. After the removal of all but 50 to 60 ml of PPP, the current general practice is to allow the platelet button to rest undisturbed for 60 to 90 minutes before resuspension. At this point in preparation, the platelet concentrates are then placed in a rotator to allow continuous agitation during storage at room temperature.

The rationale for the resting phase in platelet preparation comes from the
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He reported that the release of platelet enzymes was proportional to the degree of aggregate formation after the centrifugation of platelets. Platelets allowed to rest for 20 to 60 minutes before being rotated were free of aggregates and had decreased enzyme levels when compared to non-rested platelets.

More recently, Snyder et al. 14 in collaboration with our center, have found that platelets allowed to rest for 90 minutes had a lower B-TG and LDH release at 24 hour storage than platelets not allowed to rest. However, at 72 hour storage, the B-TG and LDH discharge was independent of the rest period. Katz et al. 4 have examined immediate resuspension of pheresis platelets by manual agitation and found no detrimental effect.

In previous studies, the effect of a rest period on other parameters, such as pH, morphology, blood gases, and recovery to osmotic stress during storage have not been examined. 1, 5, 9, 11, 12 Since many of these parameters have been useful in assessing the quality of stored platelets, these studies are performed to determine the effect of eliminating the rest period.

Materials and Methods

Platelet Preparation

Blood from 35 normal blood donors was collected into a closed triple bag system* containing CPDA-1 as anticoagulant. Platelet concentrates were prepared within four hours of blood collection using a centrifuge.† To prepare platelet rich plasma (PRP), the blood was centrifuged at 2800 rpm (2000 × g) for 2.7 minutes. To harvest a platelet button from PRP, the PRP was centrifuged at 4200 rpm (4500 × g) for five minutes. All but approximately 60 ml of platelet poor plasma (PPP) was transferred out to another satellite bag, leaving sufficient PPP to resuspend the platelet button.

Resuspension Protocols

These protocols are presented in table I. As seen in table I, platelet concentrates in the control group (group D) and in Group B were allowed to rest on a flat surface at ambient temperature for 90 minutes, while platelet concentrates in Group A and C were placed on the rotator immediately after preparation. Platelet concentrates in groups B and C were resuspended by gentle manual agitation prior to rotation. All PC were rotated at six rpm on an elliptical rotator‡ in an environmentally controlled chamber at 22 to 24°C.

Storage Studies

Following resuspension, platelets were stored as described for 72 hours. At various storage intervals, pH, pO₂, pCO₂, platelet morphology score, platelet recovery to osmotic stress, and cell counts were measured as previously described. 3 Platelet size distribution was measured according to the method of Holme et al. 2

Significant differences between groups were performed by one way analysis of

* PL-146, Fenwal, Deerfield, IL.
† Sorval RC-3, Sorval, Newtown, CT.
‡ Fenwal, Deerfield, IL.
TABLE II
pH, \( pO_2 \), and \( pCO_2 \) Measurements on Platelets Stored for 72 Hours

<table>
<thead>
<tr>
<th>Group</th>
<th>pH 2 Hour</th>
<th>pH 24 Hour</th>
<th>pH 72 Hour</th>
<th>( pO_2 ) 2 Hour</th>
<th>( pO_2 ) 24 Hour</th>
<th>( pO_2 ) 72 Hour</th>
<th>( pCO_2 ) 2 Hour</th>
<th>( pCO_2 ) 24 Hour</th>
<th>( pCO_2 ) 72 Hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>7.09±0.01*</td>
<td>6.79±0.43</td>
<td>6.77±0.34</td>
<td>66.9±20.7</td>
<td>18.3±5.0</td>
<td>25.3±16.1</td>
<td>58.4±6.8</td>
<td>79.3±14.6</td>
<td>62.9±22.3</td>
</tr>
<tr>
<td>Group B</td>
<td>7.06±0.04</td>
<td>6.90±0.14</td>
<td>6.74±0.27</td>
<td>46.0±13.7</td>
<td>17.8±7.3</td>
<td>22.8±12.1</td>
<td>62.8±3.8</td>
<td>83.2±19.8</td>
<td>71.1±8.6</td>
</tr>
<tr>
<td>Group C</td>
<td>7.09±0.03</td>
<td>6.80±0.14</td>
<td>6.65±0.34</td>
<td>51.2±18.4</td>
<td>19.2±6.1</td>
<td>20.3±6.6</td>
<td>62.6±5.3</td>
<td>84.1±15.2</td>
<td>71.4±8.6</td>
</tr>
<tr>
<td>Group D</td>
<td>7.05±0.05</td>
<td>6.91±0.14</td>
<td>6.63±0.39</td>
<td>49.4±32.0</td>
<td>26.1±29.3</td>
<td>37.4±26.7</td>
<td>65.0±5.1</td>
<td>82.3±17.5</td>
<td>76.8±18.2</td>
</tr>
</tbody>
</table>

*Mean ± 1 S.D.

\( p > 0.05 \) in all comparisons between groups.

Results

During the storage period, there was no difference between the groups and the volume of the concentrate (data not shown). The volume of PRP in all concentrates decreased by about 20 percent after 72 hours of storage because of repeated sampling. There was also no statistically significant change in platelet count over the storage period and no statistically significant difference between the groups at any time; mean platelet counts ranged from 0.89—1.2 \( \times 10^6 \) per \( \mu L \).

pH During Storage

As shown in table II, the mean pH at \( T = 2 \) for the four groups ranged from 7.05 to 7.09. A decrease in pH occurred during storage in all groups. However, there were no statistically significant differences in pH between the groups at any storage interval.

Blood Gases

\( pO_2 \) and \( pCO_2 \) values during storage are shown in table II. In all groups, there was an initial decline in \( pO_2 \) at 24 hours followed by an increase in \( pO_2 \) at 72 hours. Initially, \( pCO_2 \) increased (at \( T = 24 \) hours) but subsequently declined at 72 hours storage in all the groups. There was no statistically significant difference between the groups for \( pO_2 \) or \( pCO_2 \) at any time.

Platelet Morphology During Storage

Morphology scores for each group at various storage intervals are shown in table III. While morphology declined in each group, there were no statistically significant differences in morphology scores between the groups at any storage interval.

Recovery to Osmotic Stress During Storage

The mean ± SD recovery to osmotic stress after 72 hours of storage was as follows: group A = 53.9 ± 15.5 percent, group B = 60.06 ± 6.7 percent, group C = 59.0 ± 13.8 percent, and group D = 50.3 ± 11.2 percent. There were no statistically significant differences between the groups.

Median Platelet Volume During Storage

Median platelet volume was measured at \( T = 0 \) and \( T = 72 \) for each group. The
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Variation in the Median Platelet Volume Over 72 Hours of Storage

<table>
<thead>
<tr>
<th>Storage Time (Hours)</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate</td>
<td>6.4±1.0t</td>
<td>6.6±0.6</td>
<td>7.3±0.9</td>
<td>6.9±0.8</td>
</tr>
<tr>
<td>72</td>
<td>6.4±1.4</td>
<td>6.6±1.0</td>
<td>7.7±1.2</td>
<td>7.4±1.9</td>
</tr>
</tbody>
</table>

* n = Number of samples.
† Mean platelet volume in fl. ± 1 S.D.
p > 0.05 for comparisons between groups.

values are shown in table IV. Again, no statistically significant differences were observed.

Discussion

The purpose of this study was to determine what effect, if any, the elimination of the resting phase during platelet concentrate preparation and resuspension would have on subsequent storage of platelet concentrates. Previous studies have examined the release of platelet constituents during platelet preparation. A number of other in vitro parameters have been studied to delineate further this aspect of platelet preparation. Four resuspension methods have been investigated, two of which utilized a rest period and two which did not. One group of platelet concentrates was resuspended after the rest period with gentle manual agitation while the other group of PC was simply placed on a mechanical rotator after the rest period. Similarly, in platelet concentrates in which no rest period was allowed, one group was resuspended manually prior to rotation and the other group simply placed on a rotator.

Our studies show that the pH was well maintained during 72 hours of storage, regardless of how platelets were resuspended. Platelet morphology and recovery to osmotic stress were also well maintained over the storage interval. Changes in pO₂ and pCO₂ were independent of resuspension method and are similar to those reported previously. Volume distribution curves were also comparable in all four groups.

These results show that there are no discernable ill effects of omitting the resting phase from platelet preparation and no appreciable benefit to manual agitation prior to rotation.

The rationale for using a rest period after platelets are harvested has been to facilitate a smooth resuspension of platelets. Although aggregates were not quantitated in platelet concentrates, visual inspection of platelet concentrations after preparation and during storage showed no differences among the groups for the presence of gross aggregates. In addition, in vitro tests done during storage showed platelets to be of similar quality for all resuspension methods. These findings suggest that any one of the protocols used for resuspension may be suitable for platelet preparation. The protocol that eliminates the rest period and does not require manual agitation is desirable for logistical reasons. This would simplify the preparation protocol as well as allow the availability of processed platelets sooner than if a 90 minute rest period was used. Our studies were confined to the assessment of in vitro properties of platelets during storage; studies of the in vivo hemostatic effectiveness of platelets resuspended by different methods are indicated.

References


